

CLAIMS

What is claimed is:

1. A method for binding an exogenous molecule to a binding site, wherein the binding site is located within a region of interest in cellular chromatin, wherein the 5 method comprises:
  - (a) identifying an accessible region within the region of interest;
  - (b) identifying a target site for the exogenous molecule within the accessible region; and
  - (c) introducing the exogenous molecule into the cell;
- 10 whereby the exogenous molecule binds to the binding site.
2. The method according to claim 1 wherein the cellular chromatin is in a chromosome.
3. The method according to claim 1 wherein the accessible region is a nuclelease hypersensitive region.
- 15 4. The method according to claim 1, further comprising the step of:
  - (d) testing for binding of the exogenous molecule to the binding site.
5. The method according to claim 4, wherein testing is by a method selected from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.
6. The method according to claim 1, wherein the exogenous molecule is a 20 protein.
7. The method according to claim 6 wherein the protein performs a process selected from the group consisting of replication, recombination, integration, DNA repair, transcriptional regulation and chromatin remodeling.
8. The method according to claim 6 wherein the protein is used for detection 25 of a target sequence.
9. The method according to claim 7, wherein the protein is a transcription factor.

10. The method according to claim 9, wherein the transcription factor is a zinc finger protein (ZFP).

11. The method according to claim 6 wherein the protein is encoded by an exogenous nucleic acid introduced into the cell.

5 12. The method according to claim 1, wherein the cell is a eukaryotic cell.

13. The method according to claim 12, wherein the cell is a plant cell.

14. The method according to claim 12, wherein the cell is a mammalian cell.

15. The method according to claim 14, wherein the cell is a human cell.

16. The method according to claim 1, wherein the binding site is in a coding 10 region.

17. The method according to claim 1, wherein the binding site is in a non-coding region.

18. The method according to claim 10, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

15 each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

19. The method according to claim 1, wherein the exogenous molecule is introduced into the cell by a method selected from the group consisting of lipid-mediated 20 gene transfer, electroporation, direct injection, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran mediated transfer, and viral vector-mediated transfer.

20. The method according to claim 11 wherein the nucleic acid is introduced 25 into the cell by a method selected from the group consisting of lipid-mediated gene transfer, electroporation, direct injection, particle bombardment, calcium phosphate co- precipitation, DEAE-dextran mediated transfer ,and viral vector-mediated transfer.

21. A method for binding a ZFP transcription factor to a binding site, wherein the binding site is located within a region of interest in cellular chromatin, wherein the method comprises:

(a) identifying an accessible region within the region of interest;

5 (b) identifying a zinc finger protein (ZFP) binding sequence within the accessible region;

(c) designing a ZFP to bind to the binding sequence; and

(d) introducing the ZFP into the cell;

whereby the ZFP binds to the binding site.

10 22. The method according to claim 21 wherein the ZFP is introduced into the cell by introducing a DNA construct encoding the ZFP into the cell under conditions in which the construct expresses the ZFP.

23. The method according to claim 21 wherein the cellular chromatin is in a chromosome.

15 24. The method according to claim 21 wherein the accessible region is a nuclease hypersensitive region.

25. The method according to claim 21 further comprising the step of:

(e) testing for binding of the ZFP to the binding site.

26. The method according to claim 25 wherein testing is by a method selected 20 from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.

27. The method according to claim 21, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

25 at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

28. The method according to claim 21, wherein the ZFP is selected by phage display.

29. The method according to claim 21, wherein the ZFP is selected *in vivo*.

30. A method for identifying a binding site for an exogenous molecule, wherein the binding site is located within a region of interest in cellular chromatin, wherein the method comprises:

- 5 (a) identifying an accessible region within the region of interest; and  
(b) identifying a target site for the exogenous molecule within the accessible region.

31. The method according to claim 30, further comprising the steps of:

- 10 (c) introducing the exogenous molecule into the cell; and  
(d) testing for the binding of the molecule to the binding sequence.

32. The method according to claim 30 wherein the cellular chromatin is in a chromosome.

33. The method according to claim 30 wherein the accessible region is a nuclease hypersensitive region.

15 34. The method according to claim 30 wherein the exogenous molecule is a protein.

35. The method according to claim 34 wherein the protein is a transcription factor.

20 36. The method according to claim 35 wherein the transcription factor is a ZFP.

37. The method according to claim 30, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

25 at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

38. The method according to claim 31 wherein the exogenous molecule is a protein and wherein the protein is introduced into the cell by introducing a DNA

construct encoding the protein into the cell under conditions in which the construct expresses the protein.

**39.** The method according to claim 31 wherein testing is by a method selected from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.

**40.** A complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 30.

**41.** A complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 31.

**42.** The complex according to claim 40, wherein the exogenous molecule is a protein.

**43.** The complex according to claim 42, wherein the protein performs a process selected from the group consisting of replication, recombination, integration, DNA repair, transcriptional regulation and chromatin remodeling.

**44.** The complex according to claim 42 wherein the protein is used for detection of a target sequence

**45.** The complex according to claim 43, wherein the protein is a transcription factor.

**46.** The complex according to claim 45, wherein the transcription factor is a zinc finger protein (ZFP).

**47.** The complex according to claim 40, wherein the cell is a eukaryotic cell.

**48.** The complex according to claim 47, wherein the cell is a plant cell.

**49.** The complex according to claim 47, wherein the cell is a mammalian cell.

**50.** The complex according to claim 49, wherein the cell is a human cell.

**51.** The complex according to claim 40, wherein the binding site is in a coding region.

**52.** The complex according to claim 40, wherein the binding site is in a non-coding region.

**53.** The complex according to claim 46, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

5 each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

**54.** A method for identifying a binding site, in a chromosome, for a ZFP transcription factor, wherein the chromosome is in a cell, wherein the method comprises:

10 (a) identifying a region of interest in the chromosome;

(b) identifying a zinc finger protein (ZFP) binding sequence within the region;

(c) designing a ZFP to bind to the binding sequence;

(d) introducing the ZFP into the cell; and

15 (e) testing for the binding of the ZFP to the binding sequence by chromatin immunoprecipitation;

wherein, if binding is detected in step (e), a binding site is identified.

**55.** A method for identifying a binding site in cellular chromatin for a ZFP transcription factor, wherein the method comprises:

20 (a) identifying a region of interest in the cellular chromatin;

(b) identifying a zinc finger protein (ZFP) binding sequence within the region;

(c) designing a ZFP to bind to the binding sequence;

(d) designing a DNA construct that encodes the ZFP of step(c);

25 (e) introducing the construct into the cell; and

(f) testing for the binding of the ZFP to the binding sequence by chromatin immunoprecipitation

wherein, if binding is detected in step (f), a binding site is identified.

**56.** A cell comprising a complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 30.